Use of the prewarm method for detecting clinically significant alloantibodies in the presence of cold autoantibodies

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The prewarm (PW) method is useful for detecting and identifying clinically significant antibodies that bind to red blood cells and complement at 37°C and for avoiding antibodies that bind at temperatures less than 37°C. Antibodies that bind at temperatures less than 37°C are often cold autoantibodies that may be present in the serum of healthy individuals and are usually not clinically significant. The PW method is useful when these cold autoantibodies have a wide thermal range and interfere with standard testing methods by reacting at the 37°C and antihuman globulin test phases. When using the PW method, it is important to identify underlying, potentially clinically significant alloantibodies during pretransfusion testing to ensure the most appropriate component will be selected for transfusion.
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Principle

The prewarm (PW) method can be used to detect and identify red blood cell (RBC) antibodies that bind to antigens only at 37°C.[1] The PW method is often used in the presence of cold autoantibodies that interfere with standard testing methods and potentially mask the presence of underlying clinically significant alloantibodies. By prewarming reagents and patient serum/plasma to 37°C before testing, cold antibodies are prevented from binding, allowing for the identification of clinically significant antibodies that bind at 37°C and/or at the antihuman globulin (AHG) phase.[2] Reactions and serologic readings take place at 37°C. Hence, antibodies that react at less than 37°C are usually not detectable when using the PW method; antibodies that react at the AHG phase are detectable. Prewarming of the patient's serum/plasma and the test RBCs separately prevents binding of cold-reactive antibodies once reagents and serum/plasma are combined. Anti-IgG is used rather than polyspecific AHG to help circumvent reactions with RBC-bound complement.

Reagents/Supplies

Reagents	Supplies
▪ Normal saline or PBS	▪ 37°C incubator
▪ Anti-IgG	▪ Test tubes: 10 × 75 or 12 × 75 mm
▪ Group O reagent RBCs for antibody detection	▪ Pipettes
▪ Patient RBCs for autocontrol	▪ Centrifuge
▪ IgG-coated RBCs	▪ Agglutination viewer
▪ Group A_1, A_2, B, and O reagent RBCs	

PBS = phosphate-buffered saline; RBCs = red blood cells.

Procedural Steps

- Prewarm saline/PBS to 37°C.
- Label one test tube for each reagent RBC and A/C.
- To each labeled tube, add one drop of the respective reagent RBCs or A/C.
- Add an adequate volume of patient serum/plasma and a pipette to an appropriately labeled tube.
- Incubate tubes at 37°C for 5–10 minutes.
- Using 37°C pipette, add two drops of prewarmed patient serum/plasma to each prewarmed RBC tube and mix.
- Incubate at 37°C for 30–60 minutes.
- Wash tubes four times with 37°C saline/PBS.
- Add anti-IgG in accordance with manufacturer's directions.
- Centrifuge and observe for agglutination; grade and record results.
- Add IgG-coated RBCs to negative reactions.

PBS = phosphate-buffered saline; RBC = red blood cell; A/C = autocontrol.

Applications

The PW method is most frequently used to identify underlying antibodies in the presence of strong cold autoantibodies that react at the 37°C and AHG phases of testing. Cold autoantibodies are usually of the IgM isotype and not clinically significant. The most frequently encountered

cold autoantibody is anti-I. Anti-I is common even in the serum of healthy individuals and reacts strongly at 4°C. Other common cold autoantibodies include anti-IH, which is usually found in the serum of group A_1 individuals and reacts strongly with group O and A_2 RBCs, and anti-i, which is less common and may be seen in the serum of patients with infectious mononucleosis. These specificities are usually identified with a panel of RBCs tested after a 4°C incubation.[1] Once an antibody has been identified as one of these clinically insignificant specificities, steps should be taken to eliminate such reactivity in the AHG phase, and the PW method is one way to accomplish this.

The PW method can also be used to determine whether an antibody that reacts optimally at colder temperatures has a wide thermal range and thus may need to be considered clinically significant or to be identified as the cause of reactivity observed at 37°C. Cold autoantibodies with a wide thermal range may be found with cold agglutinin syndrome. In this setting, the antibodies bind complement and are considered clinically significant.

Cold autoantibodies can also interfere with serum/plasma testing or reverse group testing causing blood group discrepancies. A modified PW method described subsequently can be used to resolve ABO group discrepancies caused by cold autoantibodies.

Procedure

PW Method for Antibody Detection/Identification

Prewarm a container of saline/phosphate-buffered saline to 37°C. Label one test tube for each reagent/donor RBC sample to be tested, including a test tube for an autologous control (autocontrol). Label one test tube that will contain an adequate volume of patient serum/plasma for all anticipated testing and a transfer pipette. Prepare a 2–5 percent RBC suspension for each reagent or donor RBC sample and the autocontrol. Add one drop of each 2–5 percent RBC suspension to its respectively labeled tube. Place the tubes containing the RBC suspensions and the patient plasma/serum (at least two drops for each RBC to be tested and two additional drops) in a 37°C incubator for 5–10 minutes. Leave the transfer pipette in the tube containing the plasma to prewarm as well. Using the prewarmed pipette, transfer two drops of plasma to each tube containing prewarmed RBCs to be tested. Mix gently without removing from the incubator. Incubate at 37°C for 30–60 minutes. Wash and then centrifuge the tubes three to four times using 37°C warmed saline. Add anti-IgG according to the manufacturer's instructions. Centrifuge and then observe for agglutination. Grade reactions accordingly and record results. Confirm that negative AHG reactions are valid with the use of IgG-coated RBCs.

Prewarmed Serum Group Determination

Add one drop of each RBC to be tested (group A_1, A_2 (if desired), B, and O RBCs for a control) to appropriately labeled tubes. Add an adequate amount of patient serum/plasma to a separate labeled tube (at least two drops for each RBC to be tested). Warm the tubes at 37°C for 5–10 minutes, leaving the transfer pipette in the tube containing patient serum/plasma. Using the prewarmed 37°C pipette, add two drops of patient serum/plasma to each of the tubes containing reagent RBCs and gently mix. Incubate the tubes at 37°C for 1 hour. Remove the tubes and examine the settled cells for agglutination (do not centrifuge).

Limitations

The PW method should be used with caution, since decreased (clinically significant) antibody reactivity has been reported, and some weak antibodies can be missed.[3] Using room temperature saline to perform washes after incubation may help to avoid the elution of clinically significant antibodies, although strongly reactive cold autoantibodies may still react.[1] The PW method should not be used when the specificity of the antibody has not been determined, especially when the autocontrol tests are negative. Clinically significant alloantibodies such as anti-I, anti-P, and anti-Vel have been mistaken for the clinically insignificant cold-reactive antibody after the use of the PW method.[4] The PW method may not detect potentially significant complement-binding alloantibodies or hemolysis and will not detect antibodies that bind at less than 37°C. Separate tubes may be set up for a direct reading at 37°C to observe for hemolysis and agglutination.[4] Allowing these separate tubes to incubate at 37°C for 60–120 minutes and examining the settled RBCs for agglutination by resuspending the cell button may help detect these antibodies if desired.[5] When using the PW method for reverse group testing, some weak anti-A and anti-B may not be detected.

Quality Control

Quality control should be performed on all reagents and equipment used per the manufacturer's instructions for day of use. IgG-coated RBCs are used to confirm all negative AHG

reactions. If no agglutination is observed after the addition of IgG-coated cells, the indirect antiglobulin test is invalid. If agglutination is observed with the group O RBC sample when performing reverse group testing, the test is invalid, and no conclusion can be drawn regarding the ABO group.

References

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